SOME CYTOLOGICAL CHARACTERISTICS OF BROWN FAT, WITH SPECIAL REFERENCE TO THE INTERSCAPULAR LOBES IN THE SYRIAN HAMSTER AND ALBINO RAT

BY

JAE LUIS LITTRELL

A.B., Rockford College, 1939

M.S., University of Illinois, 1940

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS, 1947

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SOME CYTOLOGICAL CHARACTERISTICS OF BROWN FAT, WITH SPECIAL REFERENCE TO THE INTERSCAPULAR LOBES IN THE SYRIAN HAMSTER AND ALBINO RAT

A cytological study of the interscapular lobes of brown fat in the albino rat and Syrian hamster was made under both normal and experimental conditions. All tissues, which included those from normal adult, embryonic, immature, and experimental animals, were examined by the same technique. These methods included: (1) Formalin-acetic-alcohol fixative followed by hematoxylin-eosin and Mallory's triple stain for routine examination, (2) Feulgen technique for nuclear studies, (3) Da Fano's silver impregnation method for the demonstration of Golgi, (4) Altmann's method to demonstrate mitochondria, (5) Fleming's technique for the observation of fat, and (6) the Gomori method for the demonstration of reticular fibres.

The average weight of the major lobes of the interscapular brown fat in the albino rat was 0.372 grams, and in the hamster 0.416 grams. These averages were computed on the tissue obtained from 1600 rats and 350 hamsters. The interscapular lobes in the normal adult consist of 7 to 12 lobules. The tissue is very vascular, and with a differential stain or in injected specimens, the larger vessels in the pedicle can be seen to divide as they enter each lobe and then branch out to supply the lobules. The blood vessels branch into a fine capillary net and completely surround each of the cells within the lobules.

In fixed material these cells appear to be polyhedrons and are made up of a number of fat-like globules within the cytoplasm. These cells are 28.8 mu in diameter in the hamster and 23.5 mu in the rat. In the former the nucleus is centrally located, and much less frequently do the nuclei appear to be eccentric than in the latter. The nucleus is surrounded by a cytoplasmic net whose ramifications also surround each fat globule and merge with the cell boundary. The cytoplasm is distinctly granular in appearance, and in a Golgi preparation this apparatus is also granular. The Golgi bodies can be seen generally distributed throughout the cytoplasm, not only around or near the nucleus but around each globule also.

An observation which is brought out by the Gomori technique is that reticular fibers are actually present in the cytoplasm, so that each fat-like globule is surrounded by a fibrillar net. The network which surrounds the peripheral globules seems to be intermingled with the fibrils of the cell boundary.

The nuclei in the normal adult, both hamster and rat, gives a positive reaction with Feulgen's. There is an indication of a variation in the degree of reaction which the nuclei undergo. This variation might be accounted for by the depth of the plane of sectioning, but on the other hand there is the possibility that the chemical state of the nuclei vary sufficiently to alter the reaction. The nuclei are round or slightly ovoid and approximately 7.2 mu in diameter.

Extirpation of the adrenal gland in the rat produces marked changes in the gross and microscopic picture of brown fat. The interscapular lobes appear greatly congested, but at the same time are slightly reduced in weight; the cytoplasm is homogeneous rather than multiglobular, and the Golgi occurs in fine granules equally distributed throughout. The appearance of brown fat is also greatly changed when diabetes is induced by pancreatectomy. A marked variation occurs in the size and number of the fat globules, leading one to believe that there is an impairment of some process within which changes the formation of fatty deposits.

The cytological appearance of brown fat in male rats to which estrogen was administered in doses of 10 gamma per day for 10 days was almost identical with that of the brown fat of pancreatectomized rats. Both experimental pancreatectomy and injection of estrogen are known to affect fat metabolism. Oral administration of thiouricil, a drug which diminishes the activity of the thyroid, results in a reduction in size and an increase in number of fat droplets in the brown fat cell. It is significant that similar changes have also been described for this tissue during hibernation when the basal metabolism is minimal.

Brown fat from rats reared on a Vitamin E-deficient synthetic ration gives evidence of a general breakdown within the tissue. Fat globules that remain are irregular in size and shape, and cell boundaries are not well defined. The brown fat of hamsters that died of starvation also gives a very specific picture. For the most part the cells are devoid of fatty globules, with the exception of certain areas where there is evidence of complete cellular degeneration. The interscapular lobes decrease in size and are completely denuded of regular adipose tissue.

When hamsters are subjected to cold (4°C. for 4 days) the brown fat becomes dark brownish-red in color which might be attributed to congestion, while the globules of fat are small and compact, and the

cytoplasm is granular. There is evidence that these changes operate through the adrenal glands (Selye). Another non-specific harmful stimulus producing the "alarm reaction" is trauma. The brown fat of a traumatized hamster resembles that from animals subjected to cold.

In the hamster embryo (12 days) the fat globules appear to be formed from a single globule in the cytoplasm of the young cell, which process is contrary to the present opinion that the single fat globule in the adult adipose cell forms by the coalescence of the minute droplets present in the developing cell. In the rat embryo (15-16 days) many mitotic figures can be observed in brown fat tissue. Mitosis is not evident after the rat is three days of age.

With regard to chemical considerations of brown fat, a qualitative analysis revealed the presence of lecithin, cephalin, cholesterol, phosphorus and iron, while a quantitative analysis for potassium showed the amount to be 2.7273 milligrams per cent in contrast to 1.0606 milligrams per cent in regular adipose tissue. When radio-active disodium phosphate is fed to rats, the phosphorus turnover in brown fat, when compared to other tissue, is found to be much greater than brain, fat and striated muscle, and to resemble more closely glandular tissue than any other in its radio-activity.

The evidence set forth here and by others establishes brown fat as a tissue reacting to changes in the internal millieu which in this tissue may be conditioned by changes originating in the internal organs, such as the endocrine glands, or in response to environmental conditions. In either case, the important factor is that a change occurs, not only in the gross, but in the microscopic anatomy as well. Brown fat is a tissue with some characteristics unique to the tissue, with other characteristics shared by other groups of cells. Of particular interest is the rich blood supply to the individual cells. This condition seems to exist when the cells have a contribution to make to the organism as a whole, or when they act as an intermediary storehouse such as the liver, or as a coordinator like the adrenal. Whether or not brown fat acts in any such capacity is yet to be determined, but the experimental results certainly indicate a necessity for further investigation.

Vita

Jae L. Littrell was born in Keithsburg, Illinois, June 24, 1908. After attending public school, she entered nurse's training at St. Luke's Hospital, St. Paul, Minnesota, and graduated in 1934. She graduated from Rockford College, Rockford, Illinois with a degree of Bachelor of Arts in June, 1939. During the summer of 1937 she attended the University of Minnesota. In September, 1939, she entered the Graduate School of the University of Illinois and received a Master of Science degree from that institution in June, 1940. From 1940-1943 she was an assistant and instructor at the University of Illinois. During the summer of 1943 she studied at the Marine Biological Station at Woods Hole, Mass., and she was there during the summer of 1944 as an instructor. From 1943-1947 she was a research assistant to Dr. Carl G. Hartman in the Department of Zoology-Physiology at the University of Illinois. Honorary and professional organizations of which she is a member are Sigma Xi, Phi Sigma, Sigma Delta Epsilon, A.A.A.S., and Socratic.

Publications consist of:

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